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16. ABSTRACT

Egg, embryo, fry , and swim-up stages of steelhead trout (Salmo gairdneri) were exposed to water at total gas saturation levels ranging from 130 to 115%. Eggs, embryos, and newly hatched fry were not affected at 126.7%, but at about day 16 posthatch when the fish began swimming up deaths occurred rapidly, and at the end of the test post button-up mortality ranged from 99% at 126.7% saturation to 45% at 115.3% saturation. Bubbles in the mouth, gill cavity, and yolk sac caused flotation and severe respiratory difficulties. Rupture of yolk-sac membranes also caused death. No differences were noted in survival between fish exposed from egg to fry, and those exposed only from swim-up to fry stage. In summary, there were differences in susceptibility among steelhead life stages to air-supersaturated water; eggs, embryos, and pre-swim-up larvae were more resistant than swim-up and later fry stages.

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Egg, embryo, fry, and swim-up stages of steelhead trout (*Salmo gairdneri*) were exposed to water at total gas saturation levels ranging from 130 to 115%. Eggs, embryos, and newly hatched fry were not affected at 126.7%, but at about day 16 posthatch when the fish began swimming up deaths occurred rapidly, and at the end of the test post button-up mortality ranged from 99% at 126.7% saturation to 45% at 115.3% saturation. Bubbles in the mouth, gill cavity, and yolk sac caused flotation and severe respiratory difficulties. Rupture of yolk-sac membranes also caused death. No differences were noted in survival between fish exposed from egg to fry, and those exposed only from swim-up to fry stage. In summary, there were differences in susceptibility among steelhead life stages to air-supersaturated water; eggs, embryos, and pre-swim-up larvae were more resistant than swim-up and later fry stages.

Key words: trout, steelhead, supersaturation, gas-bubble disease, *Salmo gairdneri*, temperature, fish culture

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Nous avons exposé des truites steelhead (*Salmo gairdneri*) aux stades d'oeuf, d'embryon, d'alevin commençant à nager vers le haut («swim-up») et d'alevin à la résorption de la vésicule à de l'eau dont les niveaux de saturation en gaz totaux étaient de 130 à 115%. Les oeufs, les embryons et les alevins nouvellement éclos ne sont pas affectés à 126.7%, mais au jour 16 environ après l'éclosion, alors que les poissons commencent à nager vers le haut, les mortalités se produisent à un rythme rapide, et à la fin de l'essai le nombre de poissons morts (ventre en l'air) varie entre 99% à 126.7% et 45% à 115.3% de saturation. La présence de bulles d'air dans la bouche, la cavité branchiale et la vésicule font que les poissons ont beaucoup de difficulté à flotter et à respirer. Une autre cause de mortalité est la rupture des membranes de la vésicule. Nous n'avons pas observé de différences dans la survie des poissons exposés depuis l'oeuf jusqu'au stade d'alevin à la résorption de la vésicule et celle des poissons exposés depuis le stade de nage vers le haut à celui d'alevin à la résorption de la vésicule. En résumé, il existe des différences entre les divers stades biologiques de la truite steelhead quant à leur sensibilité à l'eau sursaturée d'air. Les oeufs, les embryons et les alevins aux stades précédant celui de la nage vers le haut sont plus résistants que les alevins qui commencent à nager vers le haut et les alevins plus âgés.

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FISH culture often requires heating water in hatching and rearing facilities to increase fish development rates. The practice can supersaturate water with dissolved gases, primarily nitrogen and oxygen, due to lower gas solubility at higher temperatures. If aeration-stripping techniques are not used to remove excess gases a potential hazard is created for developing fry. When the temperature of air-saturated water is heated 9 deg C the gases are then at 122% saturation. Embody (1934) found that raising the temperature more than 5 deg C (>111% saturation) did not affect trout eggs but the resulting fry developed gas-bubble disease. Yolk sacs became distended with gas causing fry to float at the water surface. The condition was corrected by aerating the water with a series of baffles placed at the head of the rearing trough. Rucker and Hodgeboom (1953) showed that dissolved nitrogen gas above 115% created excessive mortality in trout fry and fingerlings. Additionally, fish were more susceptible to parasitic diseases than those held at 108% N_2. Rucker and Kangas (1974) showed gas levels up to 128% total gas pressure had no effect on survival to hatching of coho (*Oncorhynchus kisutch*) or chinook (*O. tshawytscha*) salmon eggs. Meekin and Turner (1974) also showed chinook eggs were not affected at 122% N_2 but steelhead trout (*Salmo gairdneri*) eggs incubated at the same level had 50% mortality before hatching.

Objectives of this study were to evaluate effects of several concentrations of supersaturated water on survival of egg-through-fry stages of steelhead trout, to compare survival of fish exposed from egg through fry with those exposed only from swim-up (when fish leave gravel and enter the water column) through fry, and determine comparative sensitivity of different life stages.

Materials and methods — Supersaturated water was produced and percent saturation controlled by compressed air in water under pressure (Nebeker et al. 1976). Supersaturated water was introduced at the top of an inclined, partitioned glass trough where it cascaded down over partitions and was serially stripped of excess gas. Different concentrations of supersaturated water were removed at points along the trough and delivered to artificial streams and aquaria.

One hundred newly fertilized eggs (4 h postspawning) were placed in each of 12 circulating test streams. Stream channel bottoms were 10 cm wide with water 8 cm deep. The bottom of each channel was partitioned with wooden dowels to form protected yet well-aerated areas where eggs could develop, and to provide a support medium for the developing alevins. Test temperatures remained near 10°C, with water velocity at channel surface near 5 cm/s. Mean gas saturations were from 115.4 to 126.7%. Control streams were maintained near 100% (100–102%) saturation. The resulting fry were fed Oregon Moist food diet after swim-up and were reared at test saturation levels until they were 8 wk old. Fish were blotted, weighed, and measured after testing.

Fish from the same batch of eggs were also hatched and reared in unsupersaturated water and transferred to supersaturated water 2 days prior to swim-up to evaluate differences in survival between fish exposed to supersaturated water from egg through swim-up and those not exposed during early developmental stages. Ten fish with approximately half the yolk sac absorbed were transferred to each concentration of supersaturated water. Exposure in 8-L aquaria continued for 40 days when the test was terminated and survivors weighed and measured.

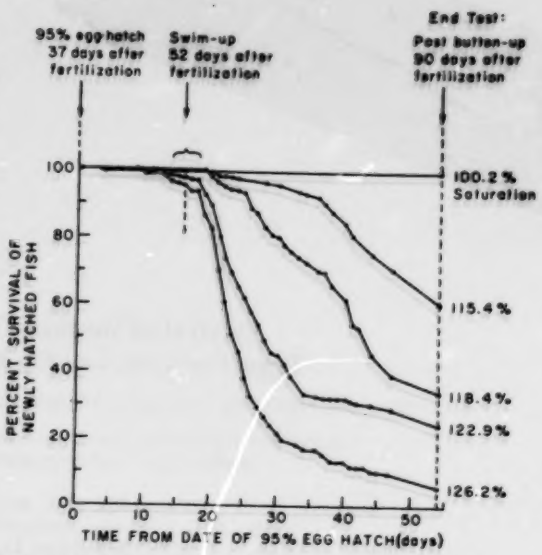

FIG. 1. Survival of steelhead trout (*Salmo gairdneri*) fry in air-supersaturated water at 10°C. Each line represents mean of two replicate groups of fish at mean saturation levels shown.

A Van Slyke gas analyzer (Beiningen 1973), a Weiss saturometer (Nebeker et al. 1976), a gas chromatograph, and Winkler dissolved oxygen determinations were used to measure total gas saturation. Dissolved oxygen and other water chemistry analyses were conducted according to methods of APHA et al. (1971). Test water was relatively soft and had the following mean chemical characteristics: hardness (as $CaCO_3$), 31 mg/L; alkalinity (as $CaCO_3$), 23 mg/L; ph, 7.1.

Results — Exposure from egg to fry — Embryos and newly hatched larvae survived 126.7% saturation, the highest level tested. However, at swim-up, 52 days after fertilization and start of exposure (16 days posthatch), death began to occur rapidly and continuously for the next 40 days until test termination. At the end of the test 45% of the juvenile fish were dead at 115.3%, 67% were dead at 118.4%, 76% were dead at 122.9%, and 99% were dead at 126.7% (Fig. 1). Survival, but not growth, of young steelhead trout was reduced at higher supersaturation levels. A wide range of tolerance to gas-bubble disease in trout was apparent. Fish surviving high gas levels often did as well as those at lower concentrations, as shown by mean weights and lengths of survivors (Table 1).

TABLE 1. Survival and growth of viable steelhead trout hatched and reared from 100 eggs (4 h after fertilization) to fingerlings after 90 days exposure in air-supersaturated water at 10°C. *Fish accidentally killed.

Test tanks	Total air percent saturation (Mean ± SD)	Surviving fish: Mean wet weight (g)	Surviving fish: Mean fork length (cm)	Surviving fish: Survival (%)
1	100.2±1.6	0.40	3.69	99
2	100.2±1.5	0.40	3.63	100
3	126.7±2.4	0.60	4.00	1
4	125.7±3.3	0.35	3.40	10
5	123.8±3.0	0.17	2.78	24
6	122.0±3.1	0.31	3.30	24
7	121.2±2.4	0.43	3.68	12*
8	120.9±1.6	0.41	3.57	4*
9	118.3±1.9	0.39	3.54	35
10	118.4±1.6	0.46	3.64	33
11	115.5±1.7	0.35	3.43	68
12	115.3±2.1	0.35	3.55	55

TABLE 2. Survival and growth of viable steelhead trout hatched and reared just prior to (2 days) swim-up in unsupersaturated water and then exposed as swim-ups to supersaturated water at 10°C for 40 days.

Test tanks	Total air percent saturation (Mean ± SD)	Surviving fish: Mean wet weight (g)	Surviving fish: Mean fork length (cm)	Surviving fish: Survival (%)
1	93.0±1.3	0.44	3.82	100
2	92.3±1.1	0.43	3.72	100
3	130.4±2.3	—	—	0
4	129.8±1.8	0.12	2.70	20
5	127.5±2.5	0.23	3.03	30
6	127.8±1.7	0.38	3.50	10
7	125.8±2.4	0.23	3.05	20
8	126.0±1.3	0.25	3.15	20
9	123.1±2.0	0.34	3.45	20
10	123.2±1.6	0.30	3.15	20
11	120.0±1.9	0.34	3.33	30
12	119.6±1.1	0.47	3.85	20

Exposure from swim-up to fry — Fish reared in unsupersaturated water and tested in supersaturated water as swim-ups had similar survival to fish exposed to supersaturated water from egg through fry. This suggests there was no acclimation from exposure as egg and larvae to supersaturated water (Table 2). Fish developed bubbles in the mouth and gill cavity after 1 day of exposure at 130%, and in the yolk sac, causing buoyancy problems by the second day. Bubbles ½ size of the yolk sac occurred at 130% but bubbles were also present at 120% saturation. Many fish floated on the water surface, unless disturbed, and swimming became progressively more difficult due to increased size and number of bubbles in the yolk sac, mouth, and gill cavity. Signs of gas-bubble disease, including exophthalmia (popeye), yolksac rupture, and emphysema (bubbles in tissue) on the head, jaw, and opercles became more evident as tests progressed. As when fish were exposed as eggs, some fish (10%) survived and grew at high saturation levels and displayed a wide range of tolerance to gas-bubble disease.

Discussion — Our study shows newly hatched fry, prior to swim-up, are more tolerant to air-supersaturated water than later stages. Larval fish tolerated 126% saturation for 2 wk with few ill effects (Fig. 1). However, as growth and development progressed to swim-up bubbles began appearing in the mouth, gill cavity, and yolk sac. Passage of water over the gills was blocked and air bubble buoyancy prevented normal swimming and feeding, and eventually floated and trapped fish at the water surface. Cause of death in several instances was rupture and destruction of yolk sac and abdominal membranes. Rapid growth and development of gills at swim-up and more rapid uptake of gases into the blood stream may explain the occurrence of gas-bubble disease at this stage. Also the thickening and strengthening of skin and other membranes may block movement of gases causing internal bubbles. The mortality rate decreased as the fish buttoned-up (when the yolk sac was no longer visible externally and the abdominal wall closed). However, this was probably due to individual variability and survival of more tolerant fish rather than fish being more tolerant at button-up. Mortality at this stage appears similar to that shown previously (Nebeker and Brett 1976).

Because the onset of gas-bubble disease does not occur until swim-up, and egg–embryo development is not affected by saturation levels of 126%, current fish culture methods of heating water to increase embryo and larval development is probably a safe practice if temperature is reduced (and thus also supersaturation) so water is not supersaturated above 108–110% when swim-up begins. Saturation levels below 105% would be preferable. Increased aeration efficiency without loss of heat would allow higher temperature increases without affecting fish. For every 1 deg C rise in temperature water supersaturation increases by about 2.5% in the range salmonids are hatched and reared. Thus, a 10 deg C rise in temperature could supersaturate water to about 125%, a possible safe level for eggs and newly hatched sac-fry, but a lethal concentration for swim-ups and later life stages.

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